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Review

Stories about acyl chains

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Contents

1. Introduction

Lipids always seem to occur in messy mixtures that frustrate students and technicians and prompt long reveries by professors. Students ask: 'Why are there so many different lipids?', and 'Why do we have all of those different acyl chains?' Story-tellers recognize patterns in natural events and tell stories about them, and audiences often urge speakers to 'fill in missing pieces' with an enjoyable hypothesis that sometimes becomes the next version of the 'story'. I have long enjoyed Kipling's 'Just So Stories' about how the camel got his 'humph' or how the spotted jaguar learned about hunting and the armadillo began [1]. The field of lipids also provides some exotic stories not fully supported by evidence. In fact, all good research begins with wishful thinking before scientific discipline converts it into controlled inter-

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ventions that address 'How does this happen?' and 'What is the consequence of this happening?'. In the end, we affirm a superiority of controlled interventions over associative evidence for choosing the best story to tell future generations. Recalling that Pauling noted long ago that chemists are grown-ups who get paid to pursue their curiosity, we can add 'and tell good stories about them'. Let me tell you some stories about acyl chains.

I began research in the 1950s when unsaturated fatty acids were becoming regarded as 'good for you' and saturated fatty acids were regarded as 'bad'. The lipid field was just beginning to develop new thin-layer and gas-liquid chromatography materials and methods to resolve the various lipids and acyl chain mixtures. Annual meetings of the American Oil Chemists' Society helped me learn new methods from Rouser [2] and Privett [3]. We used those new techniques with radioisotopic tracers to find clues to the metabolic pathways that link different lipid components. Enzymes catalyzing those links were about to be discovered, but the pathways came first. From the first lipid conference that I attended, I remember intense discussions when Hokin reported highly labeled [^{32}P]phosphatidic acid in cells, which was not predicted from the specific activity of the glycerol phosphate that was being acylated as indicated by Kennedy. The phenomenon led eventually to the discovery of diacylglycerol kinase, receptor-activated phosphoinositidase, and signal transduction, but then it was just an exciting time for exploring new ideas about how lipids are assembled.

In those days, BBA was barely one decade old [4] and support for biochemical and biomedical research was expanding very rapidly [5]. It was the 'Time of Enzymes' with new enzymes being reported every week and new biosynthetic pathways emerging out of the fog of general awareness. Two broad questions that I wanted to answer then were: 'What do unsaturated fatty acids do that is so important for human health?' and 'Which enzymes distinguish between saturated and unsaturated acyl chains?'. However, for the past 40 years we never had a pure enzyme that esterified acyl chains, and those two questions remain incompletely answered even today. Now, I look to younger associates using the powerful new tools of molecular biology and genetics to identify and clone the individual enzymes that still resist purification.

2. Structure or function?

When I was a graduate student (1951–1954), lipids seemed to be assigned to a structural role only because they had no recognized functional role other than storing energy or forming membrane structures. At the time, lipids had their roles as mixtures with no cases of a specific lipid action (except for some undefined actions of vitamin A in vision). The story that phospholipids provide necessary barriers to compartmentalize cells and prevent mixing of subcellular regions is reasonable, but it does not address why there are so many different acyl chains to perform that role. One story claims that living systems adjust the diverse mix of acyl chains to provide optimal fluidity under diverse environmental challenges, but it does not address how the enzyme 'knows' when the membrane has enough fluidity and how the enzyme changes its selectivity to provide just what is needed. In my first invited Annual Reviews chapter [6], I included Hansen's story [7] about an elevated melting point of fat in pigs wearing woolen jackets, but a changed metabolic selectivity has never been proved. However, one of the best stories on raised lipid melting points at higher culture temperatures was Fulco's result on thermal instability of a desaturase [8,9] which thereby altered the supply of acyl chains available rather than purposefully altering the esterification selectivity. Another story about 'homeoviscous adaptation' [10] addressed the consequences of the lipid mixture in membranes more than it described the decision process made at the bond-forming stage. Membrane properties are a 'bulk phase' attribute resulting from the mixture of acyl chains that initially had interacted as individual ligands with selective catalysts. This dichotomy is the essence of lipid biochemistry. The dialog between membranes and their lipid-metabolizing enzymes [11] will continue to provide many more good stories. If we are to tell the full story, it is important to identify which metabolic process regulates the mixture of acyl chains.

3. How are acyl chains placed in glycerolipids?

In general, phospholipids have about equal amounts of saturated and unsaturated acyl chains and triglycerides have about one-third saturated and two-thirds unsaturated acyl chains [6]. Using [^{14}C]acetate to label acyl chains and [^{14}C]glycerol to monitor the glycerolipid 'backbone' showed independent turnover of acyl chains in phospholipids compared to triacylglycerols [12], and led us to direct measurement of acyl-CoA thiolester transfers during the retailoring of acyl chain contents of phospholipids [13]. Snake venom phospholipase A2 and small silica gel columns were essential tools for identifying the acids released from the 2-position and retained at the 1. By 1960, it was clear from the work of Hanahan and others that biosynthetic pathways were placing saturated acyl chains at the 1-position and unsaturated acyl chains at the 2-position of glycerolipids. Our work on the selectivity of acyltransferases advanced with two dedicated assistants, Ina Merkl and Priscilla Hart, who assembled data to show selective placement of saturated acyl chains at the 1-position and unsaturated ones at the 2-position during 'retailoring' by the phospholipid acyltransferases [14,15]. By 1965, we were finally monitoring spectrophotometric assays of selective ester-forming reactions [16] in which an enzyme actually 'cared' about whether the substrate was saturated or unsaturated – and even whether it was *cis* or *trans* [17].

By the mid-1960s, the growing research team of students and technicians at Utrecht with de Haas and van Deenen was expanding our knowledge of phospholipids as membrane components. Their energy and momentum added many articles to the newly organized BBA section on Lipids and Related Subjects [4]. At that time, analytical separations were showing reproducible patterns of molecular species of the different glycerolipids, and in Ann Arbor we were intrigued by the large shifts in lecithin species upon feeding carbohydrate after a short fast [18]. The changes seemed due to a recently reported induction of acyl chain desaturase activity [19], and they were dramatic evidence of the impact of different acyl chain supplies! However, seeing the high abundance of arachidonate (40–50%) and linoleate (25–30%) species among normal rat liver lecithins unexpectedly changed my career.

Knowing those abundances led me to suggest to Bengt Samuelsson that if our bodies were to make very much of the newly discovered prostaglandins [20], the arachidonate would probably come from tissue phospholipids. That concept led to a NSF grant for a sabbatical in Stockholm in 1967 to learn mass spectroscopy and test the role of phospholipids as prostaglandin precursors [21]. It was the first of a great set of adventures about how essential fatty acids function in human health, about which I'll say more later. Rather than moving on to acyl chain regulation of gene expression, my career path veered irretrievably toward pharmacology and preventive medicine.

My enthusiasm for a possible selective formation of certain molecular species led us to examine how acyltransferases might regulate the species abundances. Acyltransferase activity discriminated among the different acyl chains in thiol esters, but it seemed indifferent to matching the entering acyl chain to the existing acyl chain at the 1-position [22]. Lecithin synthesis was producing a partially random pattern of lecithin species that looked as if the two ester positions were filled independently of each other [18]. We considered the story that placing an unsaturated acyl chain at the 2-position was done to maintain needed fluidity, but we could not support it as the transferase activity consistently maintained the same selectivity pattern irrespective of ambient temperature or solvent polarity [23]. There was no 'intelligent adaptation' by the enzyme activity. Nevertheless, the acyltransferase selectivities measured in vitro did seem relevant to living systems when they fitted well with results observed for the in vivo acyl chain compositions of glycerolipids of erythrocytes [24] and rat liver [25]. The acyltransferases seemed to care about the acyl chain being transferred from the thiol ester to the lipid even though there was no evidence that they cared about the resultant species formed or the resultant melting point of the product.

This theme was extended in the doctoral study of Linda Slakey, who developed a way to identify separately the very different 1-acyl and 3-acyl esters of triacylglycerols [26]. She then resolved and quantitated the many triacylglycerol species in rat liver and showed that each of the 1-, 2-, and 3-positions of glycerolipids had independent, selective esterifica-

tion specificities for the different acyl chains [27,28]. These results established that while forming phosphatidic acids and diacylglycerols, the enzymes of the de novo pathway place the saturated acyl chain (mostly palmitate) at the 1-position and an 18-carbon unsaturated acyl chain (commonly oleate or linoleate) at the 2-position. Then another unsaturated acid was esterified at the 3-position to form triacylglycerols with about one-third saturated acyl chains and two-thirds unsaturated acyl chains.

During this time, Ed Hill joined the lab and set about matching information on the de novo and the retailoring pathways. Although the 18-carbon essential fatty acids are esterified at the 2-position in the de novo pathway, the 20- and 22-carbon highly unsaturated essential fatty acids (HUFA) are esterified more by the 'retailoring' pathway [29,30]. As a result, HUFA are 15–20% of the acyl chains in phospholipids, whereas they are only 3% of those in triacylglycerols. At that time, we did not use the term 'HUFA', but I have done so ever since a brief visit to Guelph in the 1980s. While telling the students there about the different selectivities for transfer of 18-carbon PUFA compared to the 'long chain highly unsaturated 20- and 22-carbon PUFA', Stan Slinger encouraged me to drop the polysyllabic term and adopt the simple acronym HUFA for this important category of acyl chains. I now recognize four general metabolic categories of acyl chain: SFA, UFA, PUFA, and HUFA.

Ed Hill and I puzzled over the fact that the de novo pathway measured with washed microsomes could place almost any acid provided at both 1- and 2-positions, making a pattern of newly formed species [30] which was rarely accumulated by tissues in vivo [18]. We saw progressively less selective acylation with increasingly purified preparations from liver slices to washed microsomes, and we wondered if the purification was generating a 'stupidity' factor. How could tissue slices be so smart and microsomes be so stupid?

Around this time, van Deenen's group in Utrecht also noted that biosynthesis favors particular combinations of acyl chains [31] and saw evidence for some selective placements during cell-free synthesis [32]. Several times between 1960 and 1980, members of my lab tried to separate the various acyltransferases from liver microsomal membranes (e.g. [33,34]), but our methods were never adequate. We recognized that the activity acylating the 1-position of glycerol 3-phosphate was very different from that acylating the 1-position of 2-acyl-phospholipids [35], and the activity esterifying the 2-position of 1-acyl-glycerol-phosphate clearly differed from that acylating other 1-acyl-glycerolipids [29], but still the individual acyltransferases in the mixture (like Sleeping Beauty's castle) remained inaccessible for 40 years.

Attempts to understand how the high selectivity in vivo for acylation of glycerophosphate could be reconciled with the apparent low selectivity in vitro led Harumi Okuyama to interesting insights into a concentration-dependent selectivity [36]. Selective acylation of 1-acyl-glycerol phosphate occurred only at very low concentrations of acceptor (a condition almost always extant for tissues in vivo but not for microsomes in vitro). Harumi's use of dose-response conditions with competing pairs of substrates proved useful again years later in assignments of acyltransferase selectivity when I visited Harumi's lab for a sabbatical [37]. Looking back, we can see how excessive loads of fatty acid flood through the de novo pathway, making abnormal 'stupid' diacyl species [18] that later are gradually retailored to customary patterns after the overload recedes.

Other labs subsequently showed that intact cells can make abnormal species with two identical acyl chains [38,39]. Such results emphasize that the customary acyl chain composition is maintained only in the presence of customary supplies of substrate. Collective experience emphasizes the plasticity and promiscuity of the de novo pathway which responds to increased supplies of acyl-CoA by acylating glycerol phosphate to form diacylglycerols (and triacylglycerols in eukaryotic cells). Such results link back to the 19th century story of Chevruel describing fat as the 'élément variable' and phospholipid as the 'élément constant', and they give a modern warning that careful attention to concentration-dependent phenomena in physiological conditions is needed to interpret apparent acyl chain selectivities.

The methylation pathway acts in enriching 18:0–20:4 species in lecithin, as first suggested from asymmetric isotope distributions [40], but the origins of the abundant 18:0–20:4 molecular species in phosphatidyl inositol remain to be told. Also, our later evidence showed similar acyl chain recognition [37]

in vitro for 18:2*n*-6 and 18:3*n*-3 and could not explain what process keeps 18:3*n*-3 from accumulating in phospholipids in vivo. Since 18:3*n*-3 is readily found in triacylglycerols, it must enter the de novo pathway, but some unknown event keeps it out of phospholipid. Much more work remains to tell this story properly.

4. What do the enzymes 'see' in their favorite acyl chains?

If enzymic selection of individual saturated and unsaturated acyl chains was deciding a cell's fate, I wondered what the bond-forming enzyme 'sees' when it 'sees' its favorite substrate. I encouraged students to 'think like the enzyme does' when approaching the question 'What do the acyltransferases recognize when they recognize an unsaturated fatty acid as being different from a saturated fatty acid?'. In answering that, we were very lucky to have the help of Frank Gunstone and his postdoctoral fellows at St Andrews. They produced a prodigious series of fatty acid isomers that we converted to acyl-CoA thiol esters. We had *cis*-ethylenic [41], *trans*-ethylenic [42], *cis*-cyclopropane [43], and acetylenic [44] bonds placed at nearly every position along the acyl chain – an excellent set to see what the acyltransferases recognized as the 'right' component.

Preparation of acceptors for acyl chain transfers was aided by an earlier interest in the biochemistry of plasmalogens with my first graduate student, Huber Warner, who characterized the configuration and enzymatic cleavage of the alkenyl ether bond [45,46] and set the stage for preparing 2-acyl substrates for acyl transfer to the 1-position. A possibility that plasmalogens have separate acyltransferases arose when we found different selectivities with 1-acyl and 1-alkenyl acceptors [47]. The phenomenon interested Keizo Waku, and he carried the plasmalogen project back to his new research program upon returning to Japan. This led later to recognition of a new path of transacylation with the productive work of his student Sugiura [48,49]. In Ann Arbor, we continued to use alkenyl derivatives to make 2-acyl substrates, although an interesting invitation led me to Memphis to review results of Muirhead with an antihypertensive polar renomedullary lipid that seemed much like an ether phospholipid [50]. He and Upjohn were hoping for a fast resolution of the chemistry of the small amount available, and my obvious advice that he share samples with Fred Snyder in nearby Oak Ridge led to the rapid results reviewed by Fred last year [51].

Acyl transfer to the 2-position strongly favored the 9-, 12- and 13-*cis*-acyl chains over the other closely related isomers [41], whereas transfer to the 1-position was surprisingly selective for the low-melting 8-, 10-, and 12-*cis*-isomers. We were pleased that in vivo distributions of specific acyl chains between the 1- and 2-positions of lecithin resembled those predicted from in vitro kinetic studies [41], but further studies brought more unexpected surprises. Transfer of the *cis*-cyclopropane acyl chains to the 1-position [43] had a nearly identical pattern to that for the *cis*-ethylenic chains, suggesting that configuration was a decisive factor. However, transfer to the 2-position differed in the absence of unsaturation. Then we saw that the pattern of transfer of the higher melting *trans*-isomers to the 2-position [42] was almost identical with that of the *cis*-isomers. However, even more surprising was the shift of one carbon atom in acyl chain isomer preference for transfer to the 1-position! Clearly, the enzymes discriminated fine structural details of the acyl chains. By the time that we tested the acetylenic CoA esters, we were prepared to see sharp selectivities in which a one-carbon offset between acetylenic and *cis*-isomers was reconciled by space-filling molecular models of the acyl chain [44,52] (note that Fig. 3 in [44] differs from Fig. 3 in [52]).

Overall, the acyltransferase activities that customarily transfer saturated, high-melting acids to the 1-position also esterified many unsaturated fatty acids, and they were sensitive to differences in acyl chain conformations irrespective of unsaturation or melting point! In contrast, the activities expected to transfer unsaturated, low-melting acids to the 2-position responded to π bonds at certain locations irrespective of configuration and melting point (reviewed in [52]). Hindsight shows that the enzymes exhibited exquisite discrimination of acyl chains, but they did not use criteria or dimensions that we humans thought would be important. We had failed to 'think like the enzymes think' about the preferred substrate.

To tell a good story, we need a clearer sense of

whether a single solvated acyl-CoA thiol ester could inform the acyltransferase active site of the probable physical property of the bulk phase lipid being produced (and also whether the enzyme would 'know' that the membrane needed to have changed contents). To better discern acyl chain orientation of the individual acyl-CoA thiol esters, we sent Ron Reitz to work with Dennis Chapman on NMR scans of solvated, dilute acyl-CoA thiol esters. Unfortunately, all dilutions that gave measurable signals also indicated a micellar 'bulk phase' environment of the acyl chains, and the damp chill of an English winter proved an unforgettable experience for a young Texan who had never learned the old-fashioned art of stoking a coal stove for overnight heat!

5. Do living cells 'care' about acyl chain chemistry?

By 1970, results with microbial mutants were providing new insights into membrane lipid biochemistry in the laboratories of Fox [53], Keith [54], Overath [55], Silbert [56], and Wakil [57]. To learn methods of microbial genetics, I spent six months in Tübingen with Ulf Henning who was doing interesting membrane studies with *Escherichia coli* [58]. Knowing that acyltransferase activities discriminate very detailed features of acyl chain chemistry prompted us to wonder 'Could such fine structural details have any consequence on cell membrane physiology?'. To answer this, Alex Keith helped us obtain auxotrophic mutants (*E. coli* and *Saccharomyces cerevisiae*) that synthesize only saturated fatty acids and grow only when supplied with an unsaturated fatty acid. As cells multiplied from the initial inoculum (prepared with adequate oleate), the incorporation of endogenous palmitate formed by the daughter cells progressively diluted the unsaturated acyl chain in membrane phospholipids until the cell membranes could no longer support further DNA replication or another cell division, and cell yields were proportional to the amount of low-melting unsaturated acid provided to the cultures [59]. At stationary phase the membrane content of the essential nutrient tended to be proportional to the melting point of the bulk phase fatty acid (i.e. inversely proportional to its contribution to fluidity) confirming reports from other labs [55–57].

The normally found oleate and palmitoleate were among the least efficient acyl chains tested, whereas several HUFA not found in yeast were the highest [59]. To quantitate a linearly additive contribution to functioning cell membranes by several unsaturated acyl chains, Bruce Holub used gas chromatography to estimate a 'functionality' factor conceptually related to excess molal volume [60]. When applied to *E. coli* auxotrophs, this technique rapidly estimates the relative fluidity contribution to the membrane of a given unsaturated acyl chain [61]. For example, John Ohlrogge's thesis work showed that when cells grew until all possible nutrient was incorporated into cell membranes, the *cis*-9-octadecenoate contributed about 30% more fluidity per acyl chain than the *cis*-11-isomer. The 15 different *cis*-acyl chains had more effective isomers with the ethylenic bond near the center of the chain [61], in close agreement with observed thermal transitions of phospholipid monolayers. Similar results occurred with the *S. cerevisiae* auxotroph, and the 5- to 11-isomers gave the most effective yields. Considering that triacylglycerol formation in eukaryotic cells can divert nutrient, the *E. coli* model may give a simpler model for membrane phospholipids. Up to this time, fluidity was a dominant aspect of acyl chain function in these cellular models, although results with the *cis*-6-isomer [61] gave a hint of surprises to come.

Growth of *E. coli* auxotrophs responded to some very fine structural differences similar to those recognized by rat liver acyltransferases. Different positional isomers of acetylenic [62] and *cis*-cyclopropane [63] isomers supported growth of the *E. coli* mutant in glucose-based medium with a sharply selective pattern independent of melting point or fluidity. Only three of 16 *cis*-cyclopropane positional isomers (8-, 9-, and 11-) and three of 16 acetylenic isomers (7-, 8-, and 10-) supported growth on glucose! Those dramatic differences in cell yields and the unexpected offset by one carbon atom were not manifested by cells grown in glycerol-based medium for which the broad pattern of growth response matched that predicted by simple fluidity considerations. Clearly, something in *E. coli* was recognizing very small structural features of acyl chains. Also, we observed that adding cyclic AMP to the glucose-based medium converted the sharply selective growth responses to a broad 'fluidity' pattern like that seen with the *cis*-

ethylenic isomers [61]. We also noted that the aberrant behavior of the 6-*cis*-ethylenic isomer was 'normalized' when cells were grown on glycerol [63]. Although we speculated at the time about catabolite repression and other regulatory actions of cAMP, modern students are better prepared to interpret how this effect occurs.

When cell growth was less than expected from the gas-chromatographic estimates of acyl chain fluidity, some nutrient acid usually remained unesterified in the medium. In the glucose medium, cells appeared to be in a 'race' to incorporate the nutrient before continuously formed endogenous palmitate made the membrane unable to support further function. The glycerol-based or cAMP-supplemented media helped cells win that race. The expected role of fluidity was being usurped by selective ligand binding considerations, and this model system did not seem to have any adaptive feedback signals to prevent impending disaster [11]. New stories remain to be told about which gene products in *E. coli* are capable of such highly selective recognition of acyl chains in forming the needed lipid esters, but once again Nature has alerted us to be cautious in projecting a human sense of purpose onto molecular processes. In mammalian systems, the ethanolamine phosphotransferase prefers diacylglycerol precursors containing 22-carbon HUFA, but it does not discriminate between *n*-3 or *n*-6 structures [64–66]. However, a slower biosynthesis of 22-carbon *n*-6 HUFA relative to *n*-3 HUFA may possibly not meet the rapid kinetic needs of synthesizing phosphatidyl ethanolamine for membrane lipoproteins during perinatal brain and retina development. In this situation, we might assign a special membrane attribute to *n*-3 HUFA when a kinetic aspect of substrate availability created the phenomenon.

6. How different are *trans*-acyl chains?

The *trans*-acyl chains have many stories telling why they may be 'bad' for living systems, and we considered whether or not the bond-forming process 'knew' that it should shun this 'bad' substrate. The 1-acyltransferase activity favored 9-, 11-, and 13-*trans*-octadecenoyl isomers over the 10- and 12-isomers [42], contrasting sharply with its opposite preference for the 8- 10-, and 12-*cis*-isomers [41]. In testing the effect of the *trans*-acids on cell growth and function in glucose media, the *E. coli* mutant would not grow on any of the isomers, although none of the *trans*-isomers impeded growth on the *cis*-9-isomer [67]. Alternatively, the eukaryotic *S. cerevisiae* grew only with the 8-*trans*-isomer (with yields only one-fifth of that with the *cis*-9-isomer), and the 4-, 6-, 7-, 11-, and 12-*trans*-acyl chains impaired growth with oleate and caused a marked accumulation of triacylglycerols in the cells [67,68]. The *trans*-isomers apparently can interfere with a basic control point in lipid metabolism and perhaps the cell cycle. After learning that growth of *E. coli* in glucose medium with cAMP permitted fluidity estimates, we found that the *trans*-9- and 11-isomers had increased effectiveness with increased cAMP, and their contribution to fluidity was about one-third to one-half that of the *cis*-isomers [69]. Both unicellular models for *trans*-isomers supporting cell functions clearly indicated that selective ligand binding events may dominate results more than 'bulk phase' fluidity, even though fluidity might be the ultimate deciding feature.

To examine the influence of *trans*-acyl chains on metabolism in rats [70], we quantitated acyl chain contents in the acyl-CoA pools and different classes of lipid in liver and heart. Even when the *trans*-octadecenoate was 46% of the fatty acids in the diet, the *trans*-acyl chain was never above 15% of acyl chains in any metabolic pool measured, and increased *cis*-18:1 accompanied the elevated *trans*-18:1. The major shift seemed due to the 45% dietary *trans*-18:1 displacing dietary 18:2*n*-6 from 53 to 5.5% of fatty acid, and the 20:4*n*-6 maintained in the rat tissue phospholipids lowered from 30 to 20%, as expected. Impact of *trans*-acyl chains on cell membrane assembly and cell physiology was more sensitively detected by the severe conditions within the auxotrophic models in which the *trans*-acyl chain is diluted only by a saturated acyl chain. The cell models illustrated the importance of substrate abundance in influencing cell physiology and gave no encouragement for the optimistic story that cells can detect an impending crisis and avoid that certain death by shifting acyl chain specificity [11]. In each case, the cells grew with what they could or they died. Such an unhappy story emphasizes the value of maintaining a proper supply of acyl chains because the synthesizing enzymes only

use what they have available – for better or worse! Humans have much more information-processing 'machinery' to make informed decisions compared to the resources of an acyltransferase. We should use our insights more rigorously when selecting our foods.

7. Influencing DNA replication

Gus Graff studied an interesting selectivity in the yeast fatty acid auxotrophs when the specific essential acyl chain supplied was apparently adequate for nuclear DNA replication but inadequate for mitochondrial DNA replication [71]. In that case, newly formed daughter cells failed to receive mtDNA, and they continued further cell divisions by producing ρ^0 'petites' that no longer respired effectively and gained energy from glycolytic use of glucose. Growth with the *cis*-12 or *cis*-13 isomer produced more than 10-fold more petites than the *cis*-9 isomer. Also, the *n*-3-acyl chain of 11,14,17-20:3 caused rapid petite formation, whereas the *n*-6-isomer (8,11,14-20:3) did not. Another HUFA, 22:6*n*-3, caused 50% petites within 10 generations. We were seeing discrimination among acyl chains that were being incorporated into functional membranes and meeting the needs for initiating replication of nuclear DNA, but not the apparently higher needs for mtDNA. It took eight years and two moves until Gus joined me in Chicago to publish this work, and we thought that the world would pass us by while using another example of petite formation seen during an unsaturated fatty acid deficiency [72].

Parallel studies by Ron Walenga noted a limited time window for providing adequate acyl chains during induction of respiratory function in yeast [73,74]. An early stage of induction required high pre-existing unsaturated acyl chains in phospholipids but not a concurrent supply of unsaturated acids. This phase may involve transcription of nuclear and mitochondrial DNA to give mRNA for synthesizing the new protein components. Then, a second phase of forming new respiratory lipoprotein complexes required a fresh supply of unsaturated fatty acid, without which the newly induced lipoprotein complexes failed to develop functioning respiratory units. In each case above, the appropriate acyl chain needed to be available at specific times in the life of the cell to form new membrane components in adequate amounts. Gus Graff also found that a lower ability of the *n*-3-acyl chains 13,16,19-22:3 or 4,7,10,13,16,19-22:6 to support induction of respiration occurred before the increased appearance of petites [71]. This model system with all genes but one being competent gave no evidence of the hoped-for changes in membrane lipid that would adaptively evade disaster. Unresolved is whether the selective acyl chain effect is due to individual acyl chains binding to protein mediators or to some inadequate bulk phase property of the synthesized lipid. A fascinating story remains to be told about how the kinetics of individual acyl chains influencing gene expression or incorporating into phospholipids can control DNA transcription and replication. However, each time that I turned attention to this puzzle, new features of essential fatty acids and eicosanoids drew me off in another direction.

Acyl chain regulation of gene expression was being advanced by Vagelos [75] just before he became immersed in management at Merck, and the recent reports by Clarke [76] and Ntambi [77] have begun to move the story along again. Also, a progressive development of genetic and molecular biological tools has provided new approaches to the link between phospholipid formation and DNA replication. Kornberg's 1988 report [78] was confirmed and extended establishing that dnaA needs a fluid acidic phospholipid to open the duplex in the oriC region [79], and normal initiations of DNA replication depend upon phosphatidyl glycerolphosphate synthase [80]. Although an acidic surface with an underlying fluid phase environment is needed [81], GM1 ganglioside can also activate. This evidence makes the story that the backbone structure of cardiolipin is isosteric with the backbone of DNA now seem fortuitous. With increasing knowledge of how cell cycle events regulate phospholipid metabolism [82], we may soon hear new stories about acyl chain chemistry and gene expression. Listen carefully for evidence resolving the dynamics of single acyl chain binding versus bulk phase fluidity aspects.

8. HUFAs and hormones

By the mid-1960s, thirty years of research on nu-

trition and physiology of essential fatty acids in rats had shown 18:3*n*-3 and 18:2*n*-6 to have similar growth-promoting actions, and their related *n*-3 and *n*-6 HUFA were also essential nutrients [83–86] (reviewed by Aas-Jorgensen [87]). In addition, low humidity or limited water supply caused unexplained differences with *n*-6 and *n*-3 fatty acids, permitting only the *n*-6 fatty acids to be optimal in the conditions employed [88], a phenomenon never carefully characterized or explained to this date. A massive series of quantitative nutrition studies by Mohrhauer and Holman [89–92] set the stage for a solid understanding of competitive hyperbolic interaction of dietary 18:2*n*-6 and 18:3*n*-3 in maintaining HUFA in tissue lipids. Then came the discovery of the conversion of HUFA to prostaglandins [20] (and later other eicosanoids) with their many hormonal functions, shifting attention away from the dietary origins of essential acyl chains in membrane structures toward the pharmacology of treating eicosanoid-mediated dysfunctions. At that time, a 'mental disconnect' began to develop as the discipline of quantitative nutrition of essential fatty acids fell back and new biomedical researchers developed a rapidly growing list of medical disorders mediated by *n*-6 eicosanoids while ignoring the fact that daily dietary intake of *n*-6 nutrients was also increasing. This will be examined in later comments.

Activation of eicosanoid synthesis from *n*-6 acids rapidly provides *n*-6 eicosanoids that act strongly at tissue receptors, whereas synthesis from *n*-3 acids more slowly provides *n*-3 eicosanoids that often have weak activity at tissue receptors. In fact, some eicosanoid receptors discriminate more strongly between *n*-3 and *n*-6 derivatives than the enzymes that handle the precursors. The fatty acid oxygenases had many unexpected kinetic features that occupied much of our time and attention in the 1970s, and my second invited Annual Reviews chapter [93] dealt with the biosynthesis and metabolism of prostaglandins. The absolute requirement for hydroperoxides in the reaction mechanism of fatty acid oxygenases provided Bill Smith with unconventional stories for his doctoral thesis describing lipid hydroperoxides as essential physiological mediators [94,95] and the oxygenases as suicidal catalysts [96,97]. Because the immediate products of the oxygenases are lipid hydroperoxides, an important autocatalytic acceleration occurs [98–101], and fatty acid oxygenases can act as intracellular amplifiers of nanomolar amounts of hydroperoxides to near micromolar levels [102]. Without this explosive positive feedback, formation of active hormone would not overcome the tonic suppression of the hydroperoxide-scavenging tissue peroxidases and the inactivating catabolic dehydrogenases.

The need for a continual steady-state level of hydroperoxide ('peroxide tone') was useful insight into eicosanoid formation at inflammatory sites, and the lab enjoyed encouragement from Upjohn, Hoffmann-LaRoche, and Pfizer in characterizing the mechanisms of reversible and irreversible non-steroidal anti-inflammatory agents [97,103,104] and seeing how peroxide tone diminished action of analgesics like acetaminophen [105,106]. It was a time filled with exciting stories about how aspirin works and how it differs from ibuprofen. It led to many later adventures that included becoming a member of the Australian Rheumatism Society and making a whirlwind lecture tour around Australia with Les Cleland. In setting the stage for such stories, Harold Cook refined our oxygen electrode system and Lenny Rome ran hundreds of oxygenase assays and defined structural features of anti-inflammatory drugs with different mechanisms of inhibition [107]. While Yoichi Tamai was studying selective placement of acetylenic acyl chains into lecithin [44], Jack Vanderhoek saw selective inhibitions of cyclooxygenase with specific acetylenic isomers [108]. Both saw evidence of the earlier-mentioned one-carbon 'frame shift' involved when aligning the *cis*- and *yne*-acyl chains. Purification of the cyclooxygenase was achieved finally when Bill Smith joined the faculty at nearby Michigan State and collaborated with another great Ann Arbor PhD student, Martin Hemler [109,110]. They provided the only situation in 40 years in which our lab could use a purified lipid metabolizing enzyme that discriminated between *n*-3 and *n*-6 acyl chains! Bill Smith has continued to produce great stories ranging from cyclooxygenase localizations with fluorescent antibodies to site-directed mutagenesis and acyl chain recognition by the cyclooxygenases.

9. *n*-3 or *n*-6? Does it matter what we eat?

From the early days, we knew that prostaglandins were rapidly formed from 20:4*n*-6 in conditions in which they were not formed from 20:5*n*-3 [111,112]. This result led us to suggest that chronic inflammatory disorders linked to overproduction of *n*-6 prostaglandins might be diminished by eating more *n*-3 eicosanoid precursors. After Hamberg and Samuelsson showed in 1975 that another *n*-6 prostaglandin derivative, thromboxane A2 [113], mediated thrombosis and consequent fatality, the inhibition of cyclooxygenase acquired more vital importance than merely decreasing inflammation. The situation led Vane's group to join in interpreting the low thrombogenic tendency in Greenlanders [114] who ate lots of *n*-3 HUFA, giving strong encouragement for a preventive medicine approach [115] to improve human health by altering the *n*-3/*n*-6 proportions in tissue eicosanoid precursors.

In Ann Arbor, we showed the ability of dietary *n*-3 acids to diminish coronary thrombosis in dogs [116] and cerebral infarctions in cats [117] as well as to influence leukotriene formation in mice [118]. We saw a clear effect of dietary *n*-3 fat to diminish the *n*-6 aggregant, thromboxane A2, and the degree of human platelet aggregation [119]. This led us to compare the different levels of dietary intake of *n*-3 and *n*-6 essential fatty acids in the USA and Japan in relation to the incidence and severity of other *n*-6 eicosanoid-mediated diseases and disorders [120]. A difference in supply of substrates rather than a different enzyme specificity probably accounts for the higher disease rates in Japanese living in Western countries compared to those in Japan [121]. My concerns for the impact of acyl chain supplies were reviewed in a third Annual Reviews chapter [122] and a book, 'Fish and Human Health' [123].

The high prevalence in the USA of chronic diseases and disorders mediated by *n*-6 eicosanoids [120,124] prompts the question 'What proportion of *n*-6 HUFA in the tissue phospholipid HUFA is optimal for health?' [125–127]. Current dietary habits in the USA maintain about 75% of phospholipid HUFA as 20:3+20:4*n*-6 [122] (and cardiovascular mortality and arthritic morbidity is severe), whereas the Japanese have traditionally maintained about 50% of phospholipid HUFA as 20:3+20:4*n*-6 (and cardiovascular mortality and arthritic morbidity are less severe) and Greenlanders had even lower proportions of *n*-6 HUFA in their phospholipid HUFA with lower incidence [128] of myocardial infarction or arthritis! Our genes and our environment permit a very wide range of dietary essential fatty acid intakes to maintain very wide proportions of *n*-6 HUFA in tissue HUFA in ways that can influence the intensity of *n*-6 eicosanoid formation and function. Because the HUFA precursors of eicosanoids originate only from what we eat, the proportions of tissue *n*-6 HUFA depend on dietary abundances. Although we are auxotrophic for the *n*-3 and *n*-6 acyl chains, we are more fortunate than the microorganisms in a test tube because we can make deliberate choices of acyl chain supply. We should not just hope that somehow our body will make protective corrections when storing the HUFA precursors of eicosanoids.

10. The last two stories

In the mid-1980s, a magical moment for me occurred when a scholarly and genial official from Pfizer came to my office with an offer that I could not refuse. A Pfizer Biomedical Research Award gave me the opportunity to apply quantitative computerized treatments in assembling our current knowledge and predict answers to two key questions: 'How are *n*-3 and *n*-6 acyl chains discriminated in vitro by purified cyclooxygenase when making prostaglandins?' and 'How are *n*-3 and *n*-6 acyl chains discriminated in vivo by human biosynthetic systems when storing prostaglandin precursors?'. Those provided the last two research papers [129,130] from my lab before I retired in 1991.

The first project was carried forward by Rich Kulmacz, who had been steadily developing increasingly sophisticated insights into the cyclooxygenase reaction mechanism [131–133], and he extended earlier models with new computer simulations to handle all recognized detailed kinetic features. The fitted constants indicated that both 20:4*n*-6 and 20:5*n*-3 were handled in almost identical ways by the enzyme with only one rate constant out of 10 being different between the two acyl chains [129]. The constant for acyl chain oxygenation (k_5) was four-fold faster for 20:4*n*-6 than that for 20:5*n*-3, another of the very

few examples of an enzyme from vertebrates discriminating between *n*-3 and *n*-6 acids. This small difference explained the greater suppression of prostaglandin formation by added peroxidase when 20:5*n*-3 is the substrate, and it gave a final explanation to the 20-year-old phenomenon of crude preparations not being active with the *n*-3 substrate under conditions in which it was with the *n*-6 [111,112]. The slower rate of prostaglandin formation from 20:5*n*-3 compared to 20:4*n*-6 makes the *n*-3 acid a weak agonist that acts as an antagonist to eicosanoid formation from 20:4*n*-6, and the subsequent binding of any newly formed *n*-3 eicosanoids also antagonizes *n*-6 eicosanoid function at the receptors. Rich continues to tell interesting stories about the oxygenase mechanism from his lab in Houston.

For the second story, just as in the early years of the lab, our work on selective acyl chain metabolism was advanced by two dedicated assistants, Anna Morris and Bozena Liebelt, who assembled the needed data. We did long-term dietary studies with rats [134] to confirm the pioneering results of Mohrhauer and Holman [89,90] and to develop empirical equations that describe quantitatively the kinetic influence of dietary acids upon the HUFA maintained in tissue phospholipids. We were surprised to learn that very similar selectivities of the synthetic systems for rats [134] and humans [130] allowed close comparisons of quantitative acyl chain information between the different species. The essential PUFA, 18:2*n*-6 or 18:3*n*-3, are maintained in tissue triacylglycerols in a linear proportion to their dietary abundance expressed as percent of daily caloric energy in quantitative agreement with another report [135]. On the other hand, the *n*-3 and *n*-6 HUFA are maintained in tissue phospholipids in a competitive, hyperbolic relationship to the dietary abundance of their PUFA precursors, as shown earlier by Mohrhauer and Holman [89,90]. Since the enzymes esterifying acyl chains into various glycerolipids do not discriminate appreciably between *n*-3 or *n*-6 chains, a clear competition exists between the two types of essential fatty acid as shown in the quantitative empirical equations [130]. The average USA intake of *n*-6 fats is now an order of magnitude greater than the earlier estimated need [125,136]. Experience with the metabolism of essential fatty acids can help us design more precisely [137,138] future clinical studies that can better balance dietary *n*-3 and *n*-6 fatty acids for people at risk for *n*-6 eicosanoid-mediated disorders.

11. Looking back

My 40-year story of competing acyl chains influencing health cannot be complete without a brief look back at the parallel 40-year occurrence of the 'cholesterol story' that included saturated and unsaturated fats, a point where my research story also began. Cholesterol was one of the few lipids easily quantitated by colorimetric assay in 1950, and higher blood levels were associated with higher mortality from cardiovascular disease. Also, crystals of cholesterol accumulate in phagocytic cells at inflammatory vascular sites, and although their role as a cause or a consequence was unproved, a story of cholesterol causing death was born. Ironically, the low incidence of cardiovascular disease among Alaskan Eskimos (who ingested an *n*-3-rich diet) was associated with consistently high blood cholesterol, showing 'the causative role of serum cholesterol in development of atherosclerosis to be somewhat dubious.' [139]. Nevertheless, associative thinking fueled the story that cholesterol caused cardiovascular mortality. This occurred during the same time that a causal role for *n*-6 eicosanoids in mediating harmful inflammatory, thrombotic, and arrhythmic events was being increasingly documented – but little noted by the general community. Somehow, the certain story of eicosanoids as major mediators of mortality has never stirred audiences as the uncertain claim of cholesterol's causal role (even though people are aware of an 'aspirin story' noted earlier). Many of my colleagues still prefer the familiar but unproved story of cholesterol's causality to the proved story of *n*-6 eicosanoids in thrombosis and arrhythmia. Unfortunately, the general belief that reducing blood cholesterol levels would reduce cardiovascular mortality stimulated many efforts to add more polyunsaturated fats to the diet (producing still higher tissue levels of the *n*-6 HUFA precursors of eicosanoid-mediated diseases).

As we learn more about prenylated proteins in inflammatory and proliferative processes such as atherosclerosis [140] we are likely to place mevalo-

nate biosynthesis in a new context relative to eicosanoid-mediated events [124] and thereby develop better rationales for preventive medicine. Recent research by Brown and Goldstein [141] clarifies the importance of cholesterol in stabilizing membrane-bound sterol regulatory element binding protein (SREBP) from proteolytic activation that would otherwise activate transcription of many lipid-forming enzymes. Tight regulation by sterols of SREBP and its cleavage-activating protein SCAP [142] maintains normal lipid compositions in cells. Any weakness in the normal suppression of the SCAP-SREBP system could enhance the formation of mevalonate, prenylated proteins, and cholesterol. Since cholesterol signals suppression of its own formation, high plasma cholesterol is a marker of inadequate negative feedback (and perhaps not the main mediator of mortality [124]). Ironically, dietary cholesterol may be helping to suppress in some individuals what otherwise might become even more serious lipid imbalances.

I hope that a future story will tell us how dietary saturated and unsaturated fats created the situation that turned into the initial 'cholesterol story'. The next stories may also tell us whether cholesterol protects us from lipid disorders by generally reducing membrane fluidity or by specific ligand binding to proteins regulating the SCAP-SREBP system. The dichotomy of discrete selective lipid ligands versus aggregate properties of lipid mixtures will continue to provide future story-tellers with new tales of mystery and adventure. I plan to enjoy them.

Like the Rime of the Ancient Mariner by Coleridge [143], the above stories about acyl chains seem far too long to sit through patiently, being a very long story of human frailty, adversity, and perseverance. As you hasten to depart from this garrulous old man, I apologize for holding your attention too long and I recall the Mariner's farewell:

> He prayeth best, who loveth best
> All things both great and small;
> For the dear God who loveth us
> He made and loveth all.

References

[1] This story can be accessed at: http://www2.shore.net/~mogget/armadil.htm.

[2] G. Rouser, A.J. Bauman, G. Kritchevsky, D. Heller, J.S. O'Brien, J. Am. Chem. Soc. 38 (1961) 544.

[3] O.S. Privett, M.L. Blank, J. Am. Chem. Soc. 41 (1964) 292.

[4] Historical reflections of that time are in the 50th Anniversary Supplement of Biochim. Biophys. Acta Vol. 1335, July 1, 1997.

[5] Historical reflections of that time are on the National Science Foundation website (http://www.nsf.gov/od/lpa/nsf50/classof52.htm).

[6] W.E.M. Lands, Annu. Rev. Biochem. 34 (1965) 313–346.

[7] V. Henriques, C. Hansen, Scand. Arch. Physiol. 11 (1901) 151–165.

[8] A.J. Fulco, J. Biol. Chem. 247 (1972) 3511–3519.

[9] D.K. Fujii, A.J. Fulco, J. Biol. Chem. 252 (1977) 3660–3670.

[10] M. Sinensky, Proc. Natl. Acad. Sci. USA 71 (1974) 522–525.

[11] W.E.M. Lands, Trans. Biochem. Soc. 8 (1980) 25–27.

[12] W.E.M. Lands, J. Biol. Chem. 231 (1958) 883–888.

[13] W.E.M. Lands, J. Biol. Chem. 235 (1960) 2233–2237.

[14] W.E.M. Lands, I. Merkl, J. Biol. Chem. 238 (1963) 898–904.

[15] I. Merkl, W.E.M. Lands, J. Biol. Chem. 238 (1963) 905–906.

[16] W.E.M. Lands, P. Hart, J. Biol. Chem. 240 (1965) 1905–1911.

[17] W.E.M. Lands, J. Am. Oil Chem. Soc. 42 (1965) 465–467.

[18] W.E.M. Lands, P. Hart, J. Am. Oil Chem. Soc. 43 (1966) 290–295.

[19] D.W. Allman, D.D. Hubbard, D.M. Gibson, J. Lipid Res. 6 (1965) 63.

[20] S. Bergstrom, H. Danielsson, D. Klenberg, B. Samuelsson, J. Biol. Chem. 239 (1964) 4006.

[21] W.E.M. Lands, B. Samuelsson, Biochim. Biophys. Acta 164 (1968) 426–429.

[22] A.E. Brandt, W.E.M. Lands, Biochim. Biophys. Acta 144 (1967) 605–612.

[23] P. Jezyk, W.E.M. Lands, J. Lipid Res. 9 (1968) 525–531.

[24] K. Waku, W.E.M. Lands, J. Lipid Res. 9 (1968) 12–18.

[25] W.E.M. Lands, M.L. Blank, L.J. Nutter, O.S. Privett, Lipids 1 (1966) 224–229.

[26] W.E.M. Lands, R.A. Pieringer, P.M. Slakey, A. Zschocke, Lipids 1 (1966) 444–448.

[27] P.M. Slakey, W.E.M. Lands, Lipids 3 (1968) 30–36.

[28] E.E. Hill, W.E.M. Lands, P.M. Slakey, Lipids 3 (1968) 411–416.

[29] E.E. Hill, W.E.M. Lands, Biochim. Biophys. Acta 152 (1968) 645–648.

[30] E.E. Hill, D.H. Husbands, W.E.M. Lands, J. Biol. Chem. 243 (1968) 4440–4451.

[31] L.M.G. van Golde, L.L.M. van Deenen, Biochim. Biophys. Acta 125 (1966) 496–509.

[32] H. VandenBosch, L.M.G. van Golde, H. Eibl, L.L.M. van Deenen, Biochim. Biophys. Acta 144 (1967) 613–623.

[33] H. Eibl, E.E. Hill, W.E.M. Lands, Eur. J. Biochem. 9 (1969) 250–258.

[34] J.E. Ellingson, E.E. Hill, W.E.M. Lands, Biochim. Biophys. Acta 196 (1970) 176–192.

[35] H. Okuyama, H. Eibl, W.E.M. Lands, Biochim. Biophys. Acta 248 (1971) 263–273.

[36] H. Okuyama, W.E.M. Lands, J. Biol. Chem. 247 (1972) 1414–1423.
[37] W.E.M. Lands, M. Inoue, Y. Sugiura, H. Okuyama, J. Biol. Chem. 257 (1982) 14968–14972.
[38] R. Sundler, B. Akesson, A. Nilsson, J. Biol. Chem. 249 (1974) 5102–5107.
[39] F.H. Chilton, R.C. Murphy, Biochem. Biophys. Res. Commun. 145 (1987) 1126–1133.
[40] M. Isozaki, A. Yamamoto, T. Amako, Y. Sakai, H. Okita, Med. J. Osaka Univ. 12 (1962) 285.
[41] R.C. Reitz, M. El-Shiekh, W.E.M. Lands, I.A. Ismail, F.D. Gunstone, Biochim. Biophys. Acta 176 (1969) 480–490.
[42] H. Okuyama, W.E.M. Lands, F.D. Gunstone, J.A. Barve, Biochemistry 11 (1972) 4392–4398.
[43] H. Okuyama, W.E.M. Lands, W.W. Christie, F.D. Gunstone, J. Biol. Chem. 244 (1969) 6514–6519.
[44] Y. Tamai, W.E.M. Lands, J.A. Barve, F.D. Gunstone, Biochim. Biophys. Acta 296 (1973) 563–571.
[45] H.R. Warner, W.E.M. Lands, J. Biol. Chem. 236 (1961) 2404–2409.
[46] H.R. Warner, W.E.M. Lands, J. Am. Chem. Soc. 80 (1963) 60–64.
[47] K. Waku, W.E.M. Lands, J. Biol. Chem. 243 (1968) 2654–2659.
[48] T. Sugiura, Y. Masuzawa, K. Waku, J. Biol. Chem. 263 (1988) 17490–17498.
[49] T. Sugiura, N. Kudo, T. Ojima, K. Mabuchi-Itoh, A. Yamashita, K. Waku, Biochim. Biophys. Acta 1255 (1995) 167–176.
[50] R.L. Prewitt, B.E. Leach, L.W. Byers, B. Brooks, W.E.M. Lands, E.E. Muirhead, Hypertension 1 (1979) 299–308.
[51] F. Snyder, Biochim. Biophys. Acta 1436 (1999) 265–278.
[52] W.E.M. Lands, in: L. Bolis, M. Karnovsky, A. Leaf (Eds.), Aberrations in Membrane Structure and Function, Alan R. Liss, New York, 1988, pp. 11–28.
[53] C.F. Fox, Proc. Natl. Acad. Sci. USA 63 (1969) 850–855.
[54] A.D. Keith, M.R. Resnick, A.B. Haley, J. Bacteriol. 98 (1969) 415–420.
[55] P. Overath, H.V. Schairer, W. Stoffel, Proc. Natl. Acad. Sci. USA 67 (1970) 606–612.
[56] D.F. Silbert, F. Ruch, P.R. Vagelos, J. Bacteriol. 95 (1968) 1658–1665.
[57] M. Esfahani, E.M. Barnes, S.J. Wakil, Proc. Natl. Acad. Sci. USA 64 (1969) 1057–1064.
[58] U. Henning, G. Dennert, K. Rehn, G. Deppe, J. Bacteriol. 98 (1969) 784–796.
[59] E.D. Barber, W.E.M. Lands, J. Bacteriol. 115 (1973) 543–551.
[60] B.J. Holub, W.E.M. Lands, Can. J. Biochem. 53 (1975) 1262–1277.
[61] J.B. Ohlrogge, E.D. Barber, W.E.M. Lands, F.D. Gunstone, I.A. Ismail, Can. J. Biochem. 54 (1976) 736–745.
[62] W.E.M. Lands, J.B. Ohlrogge, J.R. Robinson, R.W. Sacks, J.A. Barve, F.D. Gunstone, Biochim. Biophys. Acta 486 (1977) 451–461.
[63] W.E.M. Lands, R.W. Sacks, J. Sauter, F. Gunstone, Lipids 13 (1978) 878–886.
[64] H. Kanoh, K. Ohno, Biochim. Biophys. Acta 380 (1974) 199–207.
[65] Y. Masuzawa, Y. Nakayawa, K. Waku, W.E.M. Lands, Biochim. Biophys. Acta 713 (1982) 185–192.
[66] Y. Masuzawa, S. Okano, K. Waku, H. Sprecher, W.E.M. Lands, J. Lipid Res. 27 (1986) 1145–1153.
[67] G. Vandenhoff, F.D. Gunstone, J. Barve, W.E.M. Lands, J. Biol. Chem. 250 (1975) 8720–8727.
[68] G. Graff, W.E.M. Lands, Chem. Phys. Lipids 17 (1976) 301–314.
[69] Y.K. Tsao, W.E.M. Lands, Science 207 (1979) 777–779.
[70] Y. Masuzawa, M.R. Prasad, W.E.M. Lands, Biochim. Biophys. Acta 919 (1987) 297–306.
[71] G. Graff, J. Sauter, W.E.M. Lands, Arch. Biochem. Biophys. 224 (1983) 342–350.
[72] S. Marzuli, R.M. Hall, A.W. Linnane, Biochem. Biophys. Res. Commun. 57 (1974) 372–378.
[73] R.W. Walenga, W.E.M. Lands, J. Biol. Chem. 250 (1975) 9121–9129.
[74] R.W. Walenga, W.E.M. Lands, J. Biol. Chem. 250 (1975) 9130–9136.
[75] P.K. Flick, J. Chen, R.P. Vagelos, J. Biol. Chem. 252 (1977) 4242.
[76] D.B. Jump, S.D. Clarke, Annu. Rev. Nutr. 19 (1999) 63–90.
[77] A.M. Sessler, J.M. Ntambi, J. Nutr. 128 (1998) 923–926.
[78] K. Sekimizu, B.Y-M. Yung, A. Kornberg, J. Biol. Chem. 263 (1988) 7136–7140.
[79] C.E. Castuma, E. Crooke, A. Kornberg, J. Biol. Chem. 268 (1993) 24665–24668.
[80] W. Xia, W. Dowhan, Proc. Natl. Acad. Sci. USA 92 (1995) 783–787.
[81] J. Garner, E. Crooke, EMBO J. 15 (1996) 3477–3485.
[82] S. Jackowski, J. Biol. Chem. 271 (1996) 20219–20222.
[83] G.O. Burr, M.M. Burr, J. Biol. Chem. 86 (1930) 587–620.
[84] O. Turpeinen, J. Nutr. 15 (1937) 351–366.
[85] G.O. Burr, J.B. Brown, J.P. Kass, W.O. Lundberg, Proc. Soc. Exp. Biol. Med. 44 (1940) 242–245.
[86] F.W. Quackenbush, F.A. Kummerow, H. Steenbock, J. Nutr. 24 (1942) 213–224.
[87] E. Aaes-Jorgensen, Physiol. Rev. 41 (1961) 2–46.
[88] H.J. Thomasson, Nature 194 (1962) 973.
[89] H. Mohrhauer, R.T. Holman, J. Lipid Res. 4 (1963) 151–159.
[90] H. Mohrhauer, R.T. Holman, J. Nutr. 8 (1963) 67–74.
[91] H. Mohrhauer, J.J. Rahm, J. Seufert, R.T. Holman, J. Nutr. 91 (1967) 521–527.
[92] H. Mohrhauer, R.T. Holman, J. Nutr. 91 (1967) 528–534.
[93] W.E.M. Lands, Annu. Rev. Physiol. 41 (1979) 633–652.
[94] W.E.M. Lands, R.E. Lee, W.L. Smith, Ann. NY Acad. Sci. 180 (1971) 107–122.
[95] W.L. Smith, W.E.M. Lands, Biochemistry 11 (1972) 3276–3285.
[96] W.L. Smith, W.E.M. Lands, Biochem. Biophys. Res. Commun. 41 (1970) 846–851.

[97] W.L. Smith, W.E.M. Lands, J. Biol. Chem. 246 (1971) 6700–6704.
[98] H.W. Cook, W.E.M. Lands, Biochem. Biophys. Res. Commun. 65 (1975) 464–471.
[99] H.W. Cook, W.E.M. Lands, Nature 260 (1976) 630–632.
[100] M.E. Hemler, G. Graff, W.E.M. Lands, Biochem. Biophys. Res. Commun. 85 (1978) 1325–1337.
[101] M.E. Hemler, H.W. Cook, W.E.M. Lands, Arch. Biochem. Biophys. 193 (1979) 340–345.
[102] R.J. Kulmacz, W.E.M. Lands, in: H.J. Forman, E. Cadenas (Eds.), Oxidative Stress and Signal Transduction, Chapman and Hall, New York, 1997, pp. 134–156.
[103] W.E.M. Lands, Trends Pharmacol. Sci. 2 (1981) 78–80.
[104] R.J. Kulmacz, W.E.M. Lands, J. Biol. Chem. 260 (1985) 12572–12578.
[105] W.E.M. Lands, A.M. Hanel, Prostaglandins 24 (1982) 271–278.
[106] A.M. Hanel, W.E.M. Lands, Biochem. Pharmacol. 31 (1982) 3307–3311.
[107] L.H. Rome, W.E.M. Lands, Proc. Natl. Acad. Sci. USA 72 (1975) 4863–4865.
[108] J. Vanderhoek, W.E.M. Lands, Biochim. Biophys. Acta 296 (1973) 374–381.
[109] M. Hemler, W.E.M. Lands, W.L. Smith, J. Biol. Chem. 251 (1976) 5575–5579.
[110] M.E. Hemler, W.E.M. Lands, J. Biol. Chem. 255 (1980) 6253–6261.
[111] C.B. Struijk, R.K. Beerthuis, H.J.J. Pabon, D.A. Van Dorp, Recl. Trav. Chim. 85 (1966) 1233.
[112] W.E.M. Lands, P.R. LeTellier, L.H. Rome, J.Y. Vanderhoek, Adv. Biosci. 9 (1973) 15–27.
[113] M. Hamberg, B. Samuelsson, Proc. Natl. Acad. Sci. USA 72 (1975) 2994–2999.
[114] J. Dyerberg, H.O. Bang, E. Stofferson, S. Moncada, J.R. Vane, Lancet 1 (1978) 117.
[115] W.E.M. Lands, B. Pitt, B.R. Culp, Herz 5 (1980) 34–41.
[116] B.R. Culp, W.E.M. Lands, B.R. Lucchesi, B. Pitt, J. Romson, Prostaglandins 20 (1980) 1021–1031.
[117] K.L. Black, B.R. Culp, D. Madison, O.S. Randall, W.E.M. Lands, Prostaglandins Med. 3 (1979) 257–268.
[118] R.C. Murphy, W.C. Pickett, B.R. Culp, W.E.M. Lands, Prostaglandins 22 (1981) 613–622.
[119] W.E.M. Lands, B.R. Culp, A. Hirai, R. Gorman, Prostaglandins 30 (1985) 819–825.
[120] W.E.M. Lands, T. Hamazaki, K. Yamazaki, H. Okuyama, K. Sakai, Y. Goto, V.S. Hubbard, Am. J. Clin. Nutr. 51 (1990) 991–993.
[121] S. Mizushima, E.H. Moriguchi, Y. Nakada, Y. Nara, K. Murakami, R. Horie, Y. Moriguchi, G. Mimura, Y. Yamori, Hypertens. Res. 15 (1992) 45–55.
[122] W.E.M. Lands, Annu. Rev. Nutr. 11 (1991) 41–60.
[123] W.E.M. Lands, Fish and Human Health, Academic Press, Orlando, FL, 1986, pp. 1–170.
[124] W.E.M. Lands, in: K.T. Lee, Y. Oike, T. Kanazawa (Eds.), The Third International Conference on Nutrition in Cardio-Cerebrovascular Diseases, The New York Academy of Sciences, 1993, Vol. 676, pp. 46–59.
[125] W.E.M. Lands, in: A. Bendich, C.E. Butterworth (Eds.), Preventive Nutrition: The Role of Micronutrients in Health and Disease, Marcel Dekker, New York, 1991, pp. 9–34.
[126] W.E.M. Lands, in: A. Simopoulos, R. Kifer, R. Martin, S.E. Barlow (Eds.), World Review of Nutrition and Diet, Karger, Basel, 1991, pp. 177–194.
[127] W.E.M. Lands, in: Proceedings from the Scientific Conference on Omega-3 Fatty Acids in Nutrition, Vascular Biology, and Medicine, Am. Heart Assoc., 1994, pp. 1–8.
[128] N. Kromann, A. Green, Acta Med. Scand. 208 (1980) 401–406.
[129] R.J. Kulmacz, R.B. Pendleton, W.E.M. Lands, J. Biol. Chem. 269 (1994) 5527–5536.
[130] W.E.M. Lands, B. Libelt, A. Morris, N.C. Kramer, T.E. Prewitt, P. Bowen, D. Schmeisser, M.H. Davidson, J.H. Burns, Biochim. Biophys. Acta 1180 (1992) 147–162.
[131] R.J. Kulmacz, W.E.M. Lands, Prostaglandins 25 (1983) 531–540.
[132] R.J. Kulmacz, W.E.M. Lands, Prostaglandins 29 (1985) 175–190.
[133] P.J. Marshall, R.J. Kulmacz, W.E.M. Lands, J. Biol. Chem. 262 (1987) 3510–3517.
[134] W.E.M. Lands, A.J. Morris, B. Libelt, Lipids 25 (1990) 505–516.
[135] W.A. Van Staveren, P. Deurenberg, M.B. Katan, J. Burema, L.C. de Groot, M.D. Hoffmans, Am. J. Epidemiol. 123 (1986) 455–463.
[136] W.E.M. Lands, Food Technol. 53 (1999) 120.
[137] W.E.M. Lands, Am. J. Clin. Nutr. 61 ((Suppl.)) (1995) 721S–725S.
[138] W.E.M. Lands, in: J.Y. Vanderhoek (Ed.), Frontiers in Bioactive Lipids, Plenum Press, New York, 1996, pp. 23–30.
[139] C.G. Wilber, V.E. Levine, Exp. Med. Surg. 8 (1950) 422–425.
[140] R. Ross, New Engl. J. Med. 340 (1999) 115–126.
[141] M.S. Brown, J.L. Goldstein, Cell 89 (1997) 331–340.
[142] A. Nohturfft, M.S. Brown, J.L. Goldstein, Proc. Natl. Acad. Sci. USA 95 (1999) 12848–12853.
[143] This poem by S.T. Coleridge is at http://www.selfknowledge.com/rime10.htm.